(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Thioredoxin peroxidase (TPX) Activity Assay Kit

Catalog No.: BC00155

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Thioredoxin peroxidase (TPX) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, fungi, tissues
Assay Type	Quantitative
Detection Instrument	Ultraviolet Spectrophotometer (optimal detection wavelength 240 nm)

Product Introduction

TPX belongs to the peroxidase family and exerts its antioxidant effects in vivo primarily by reducing hydrogen peroxide and some hydroperoxides, similar in function to GPX. It is also one of the key enzymes in the glutathione redox cycle. TPX is ubiquitous in various organisms, such as yeast, plants, animals, protozoa, parasites, bacteria, and archaea, and is highly conserved evolutionarily. TPX is closely related to the regulation of cell proliferation, differentiation, apoptosis, and tumorigenesis. The main functions of TPX include cell detoxification, antioxidation, and regulation of hydrogen peroxide-mediated signal transduction and immune responses.

Principle

TPX catalyzes the oxidation of dithiothreitol (DTT) by H_2O_2. The absorption wavelength of H_2O_2 is 240 nm. By measuring the rate of decrease in absorbance at 240 nm and subtracting the H_2O_2 decomposed by catalase (CAT) from the control, the TPX activity can be calculated. Therefore, this kit can simultaneously determine the TPX and CAT activities of a sample.

Components

Components	Size	Storage
Reagent 1	Liquid × 1 bottle	RT
Reagent 2	Liquid × 1 bottle	-20℃
Reagent 3	Liquid × 1 bottle	2~8℃

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Tissue sample preparation: Homogenize the tissue sample at a ratio of 1:5~10 (tissue mass (g): reagent volume (mL) – it is recommended to weigh approximately 0.1g of tissue and add 1mL of reagent – in an ice bath. Centrifuge at 10000g, 4℃ for 10min, and collect the supernatant on ice for analysis.
2. Bacterial and fungal samples: Disrupt the cells by sonication in an ice bath at a ratio of 500-1000:1 (1 mL of reagent 1 is recommended for 5 million cells). The cells are 10^4 cells per volume of reagent 1. The cells are 300W, 3 seconds of sonication, 7 seconds of interval, and a total of 3 minutes. The cells are then centrifuged at 1000g for 10 minutes at 4°C. The supernatant is collected and placed on ice for testing.

Operation process

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 240 nm, and zero it with distilled water;
2. Preheat reagent 1 and reagent 2 at 37°C (mammals) or 25°C (other species) for at least 30 minutes;
3. CAT activity assay tube: Take 1 mL of quartz cuvette, add 20 μL of supernatant, 900 μL of

reagent one, and 80 μL of reagent three. Mix quickly and measure the absorbance at 240 nm for 10 s and 130 s, which are A1 and A2, respectively. ΔA_{CAT}=A1-A2;

4. Total activity assay tube: Take a 1 mL quartz cuvette, add 20 μL of supernatant, 900 μL of reagent II, and 80 μL of reagent III. Mix quickly and measure the absorbance at 240 nm for 10 s and 130 s, which are A3 and A4, respectively. ΔA_{total}=A3-A4.

Note: For a large number of samples, the CAT activity of each sample needs to be measured individually.

Result calculation

1. Calculated based on sample protein concentration

Unit definition: At 37°C or 25°C, one unit (1 U) is defined as the amount of enzyme that catalyzes 1 μmol of H_2O_2 per minute per milligram of protein.

$$\text{CAT activity (U/mg prot)} = \Delta A_{CAT} \div (\varepsilon \times d) \times V_{\text{reaction total}} \div (Cpr \times V_{\text{sample}}) \div T$$

$$= 0.573 \times \Delta A_{CAT} \div Cpr$$

$$\text{Total activity (U/mg prot)} = \Delta A_{total} \div (\varepsilon \times d) \times V_{\text{reaction total}} \div (Cpr \times V_{\text{sample}}) \div T$$

$$= 0.573 \times \Delta A_{total} \div Cpr$$

$$\text{TPX (U/mg prot)} = \text{Total activity} - \text{CAT activity}$$

2. Calculated by sample quality

Unit definition: At 37°C or 25°C, one unit (1 U) is defined as the amount of enzyme that catalyzes 1 μmol of H_2O_2 per minute per gram of tissue.

$$\text{CAT activity (U/g)} = \Delta A_{CAT} \div (\varepsilon \times d) \times V_{\text{reaction total}} \div (W \times V_{\text{sample}} \div V_{\text{sample total}}) \div T$$

$$= 0.573 \times \Delta A_{CAT} \div W$$

$$\text{Total activity (U/g)} = \Delta A_{total} \div (\varepsilon \times d) \times V_{reaction\ total} \div \left(W \times V_{sample} \div V_{sample\ total}\right) \div T$$

$$= 0.573 \times \Delta A_{total} \div W$$

$$\text{TPX (U/g)} = \text{Total activity} - \text{CAT activity}$$

3. Calculated by cell number

Unit definition: At 37°C or 25°C, one unit (1 U) is defined as the amount of enzyme that catalyzes 1 μmol of H_2O_2 per minute per 10^4 cells.

$$\text{CAT activity (U/10}^4\text{cell)} = \Delta A_{CAT} \div (\varepsilon \times d) \times V_{reaction\ total} \div \left(\text{cell number} \times V_{sample} \div V_{sample\ total}\right) \div T$$

$$= 0.573 \times \Delta A_{CAT} \div \text{cell number}$$

$$\text{Total activity (U/10}^4\text{cell)} = \Delta A_{total} \div (\varepsilon \times d) \times V_{reaction\ total} \div \left(\text{cell number} \times V_{sample} \div V_{sample\ total}\right)$$

$$\div T = 0.573 \times \Delta A_{total} \div \text{cell number}$$

$$\text{TPX (U/10}^4\text{cell)} = \text{Total activity} - \text{CAT activity}$$

Annotation

ε: The molar absorptivity of H_2O_2 at 240 nm is 4.36 × 10^4 L/mol/cm = 0.0436 L/μmol/ cm;

d: Cuvette path length (cm), 1cm;

$V_{reaction\ total}$: Total volume of the reaction system, 1000 μL = 1 x 10^{-3}L

$V_{sample\ total}$: 1 mL of the extracted solution added;

V_{sample} : Volume of supernatant added to the reaction system (mL), 20μL = 0.02mL;

T: Catalytic reaction time, 2 min;

Cpr: Protein concentration in the supernatant (mg/mL), which needs to be determined separately. It is

recommended to use our company's BCA protein content assay kit (BC00006).

W: Sample mass, g;

Cell count: 10^4.

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.